

Review

Mast Cell and Immune Inhibitory Receptors

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Modulation by balancing activating and inhibitory receptors constitutes an important mechanism for regulating immune responses. Cells that are activated following ligation of receptors bearing immunoreceptor tyrosine-based activation motifs (ITAMs) can be negatively regulated by other receptors bearing immunoreceptor tyrosine-based inhibition motifs (ITIMs). Human mast cells (MCs) are the major effector cells of type I hypersensitivity and important participants in a number of disease processes. Antigen-mediated aggregation of IgE bound to its high-affinity receptor on MCs initiates a complex series of biochemical events leading to MC activation. With great detailed description and analysis of several inhibitory receptors on human MCs, a central paradigm of negative regulation of human MC activation by these receptors has emerged. *Cellular & Molecular Immunology*. 2004;1(6):408-415.

Key Words: MC, ITIM, ITAM

Introduction

Vertebrate immune system has the ability to maintain a precarious equilibrium between the extremes of reactivity and quiescence and to maintain control over cell cytotoxicity. These various forms of immune responses triggered by antigen recognition and the communication among immune cells are generally mediated by membrane bound receptors, through the cross talk of various receptors expressed on the same cells and immune cell-cell interaction(s). In most cases, immune cells are activated as a result of productive interactions between ligands and immunoreceptors followed by recruitment of cytoplasmic protein tyrosine kinases, which transmit a unique phosphorylation signal. In recent years, there has been an increasing interest in elucidating the processes involved in the negative regulation of immunoreceptor-mediated signal transduction. Evidence is accumulating that immunoreceptor signaling is regulated by complex and highly regulated mechanisms that involve receptors, protein tyrosine kinases, protein tyrosine phosphatases, lipid phosphatases, ubiquitin ligases, and inhibitory and activating adaptor molecules. It becomes clear that the interplay of activation and inhibition is necessary to modulate immune responses and the inhibitory machinery is crucial for maintaining normal immune cell homeostasis. Loss of inhibitory signaling may result in autoreactivity

and may lead to autoimmune diseases (1-3).

The first important insight into the mechanisms of inhibitory signaling came from observations that recruitment of the SH2-domain-containing tyrosine phosphatase 1 (SHP-1) to the erythropoietin receptor (EPO) caused inactivation of JAK2 and termination of proliferative signals (4). It was subsequently shown that Fc γ RIIB, a receptor for immunoglobulin G constant (Fc) region known to mediate inhibition of antigen receptor activation of B cells, could recruit a protein later known as SHP-1 as well as the closely related and ubiquitously expressed phosphotyrosine phosphatase SHP-2 through a 13-amino acid sequence (5-7). The identification of the intracytoplasmic motif containing a 13 amino acid sequence (AENTITYSLLKHP) that mediated the inhibitory effect in these studies was an important step in defining immune inhibitory receptors. This motif is now termed as immunoreceptor tyrosine-based inhibition motif (ITIM). It consists of the sequence (I/V/L/S)xYxx(L/V), where I is isoleucine, V is valine, L is leucine, S is serine, Y is tyrosine, and x is any residue. The prevailing model predicts that ITIM-containing receptors are tyrosine phosphorylated in response to immunoreceptor engagement by the Src family protein kinases (8-11). This phosphorylation creates high affinity binding sites for at least two classes of SH2 containing inhibitory signaling effectors namely SHP-1 and SH2-containing inositol phosphatases (SHIP), which are then recruited to the plasma membrane and activated through conformational changes. These activated phosphatases will dephosphorylate a variety of immunoreceptor-regulated substrates; as a result, cellular activation is inhibited (12, 13). Signaling pathways and phenotypic outcomes resulting from the molecular interaction of SHP-1 and SHIP with the ITIMs of non-overlapping subsets of inhibitory receptors are significantly

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Table 1. Immune inhibitory receptors.

Negative receptors	ITIMs	Putative activating receptor(s)	Ligands	Location
Fc γ RIIB	1	Fc γ RIII, Fc γ RIIA, Fc ϵ RI	IgG complexes	1q23-24
Siglec2, 3, 5, 6, 7, 8, 10, 11	2-5		Sialic acid	19q13.4
MAFA	1	Fc ϵ RI?		12p12-13
SIRP α	2	SIRP β	CD47	20p13
LIRs	4	ILT1, 7, 8, 6a	HLA	19q13.4
KIR	2	KIR2DS, KIR3DS	HLA	19q13.4
LAIR1	2		EP-CAM	19q13.4
CTLA-4		CD28	CD80, CD86	2q33
PD-1	1	ICOS	PD-1 ligand	2q37.3
CD72	2			9p
CD5	1			11q13
CD31	6		CD31	17q23
CD66a	2			19q13.2
NKG2A	2	NKG2C, NKG2E	HLA-E	12p13.1-2

different (14).

In contrast to immunoreceptors that contain tyrosine-based activation motif (ITAM), relatively few inhibitory receptors have been defined; up to date, less than 20 immune inhibitory receptors have been identified in human immune cell types. Inhibitory receptors can be classified into two major groups based on differences in structure; most of them are monomeric proteins that contain multiple immunoglobulin superfamily (IgSF) domains in their extracellular regions. This first group includes Fc γ RIIB, Siglec family, signal regulatory protein α (SIRP α), Ig-like transcripts/leukocyte immunoglobulin receptors (ILTs/LIRs), killer cell Ig-like receptor (KIR), PECAM-1, CEACAM-1, leukocyte-associated Ig-like receptors-1 (LAIR-1), CMRF-35H, and paired Ig-like type 2 receptor α (PILR-A). The second group of inhibitory receptors involves C-type lectin-like molecules such as mast cell function-associated antigen (MAFA), natural killer cell lectin (NKG2A), and CD72. Table 1 shows the compilation of members of the extended family of inhibitory receptors, their paired putative activating receptors, and ligands found in human immune cell types.

MCs are highly granulated cells that are widely distributed throughout the body in connective tissues and on mucosal surfaces. MCs are derived from bone marrow progenitors that migrate into the blood and subsequently into the tissues where they undergo final maturation. MCs are important effector cells in T helper 2 (Th2)-cell-dependent, immunoglobulin E-associated allergic disorders. Upon activation, MCs release a wide spectrum of mediators by exocytosis that include: (a) preformed granule-associated inflammatory mediators (histamine, neutral proteases, pre-formed cytokines, and proteoglycans); (b) lipid mediators such as prostaglandin D2 (PGD2), leukotriene (LT) B4, and LTC4; and (c) a host of proinflammatory, chemo-attractive, and immunomodulatory cytokines. The bio-activities of these mediators include bronchoconstriction,

vasodilation and tissue edema, leukocyte infiltration, collagen matrix turnover and stromal cell growth, and hyperplasia of bronchial smooth muscle.

MCs express the heterotetrameric high-affinity IgE receptor (Fc ϵ RI) which consists of α , β and γ subunits. The β and γ subunits of Fc ϵ RI contain the conserved ITAMs in their cytoplasmic tails. Cross-linking of IgE that is bound to the high-affinity receptor Fc ϵ RI with multivalent antigen, results in the aggregation of Fc ϵ RI and the secretion of pro-inflammatory and immunoregulatory mediators that can have effector, immunoregulatory, or autocrine effects. However, early data obtained from human MCs revealed that engagement of IgG receptors (Fc γ R) can promote or inhibit MC activation. Aggregation of Fc γ RI on human MCs promotes mediator release in a manner generally similar to that observed following Fc ϵ RI aggregation, whereas aggregation of Fc γ RIIB fails to influence cellular processes. However, when co-ligated with Fc ϵ RI, Fc γ RIIB becomes rapidly phosphorylated on tyrosine residues, which in turn recruits SHIP resulting in the inhibition of Fc ϵ RI-induced calcium mobilization, degranulation and cytokine production.

MC proliferation is positively regulated by the receptor tyrosine kinase kit (c-kit) through its interaction with a corresponding ligand (kit ligand). C-kit is a trans-membrane protein that belongs to the type III receptor tyrosine kinase family (15, 16). Kit ligand is believed to induce dimerization or oligomerization of its receptor, resulting in the activation of the tyrosine kinase activity of the receptor which in turn induces a rapid, but transient, tyrosine phosphorylation of several cellular proteins, including c-kit itself. A number of substrates of c-kit have been found to associate with the receptor kinase after activation, including PLC- γ , p85PI3K and SHP2 (17-19). These proteins may link with c-kit directly in various enzymatic pathways. Kit ligand activates multiple signal transduction components including PI3K, Src family

protein kinases, the Ras-Raf-MAP kinase cascade, and the JAK/STAT pathway. Over the past several years it has become increasingly apparent that MCs express a number of surface receptors that counteract MC activation. This review summarizes the common aspects of these negative regulating receptors and focuses mainly on a growing number of ITIM domain containing receptors as well as their pair of putative activating receptors that are expressed in human MCs and their role in regulating MC activation.

Fc γ RIIB

Fc γ RIIB has been studied in great details and has provided the best examples in coordinate and opposing roles displayed by activating and inhibitory receptors (20, 21). Fc γ RIIB is a type I transmembrane protein consisting of 291 amino acids and containing a signal peptide, two extracellular Ig-like domains with 3 potential N-glycosylation sites, a transmembrane region and a cytoplasmic tail (22, 23). Fc γ RIIB and Fc γ RIIA share 95% amino acid identity in their extracellular domains. Fc γ RIIB, however, contains a 13-amino acid (AENTITYSLLKHP) cytoplasmic ITIM domain which has been shown to be both necessary and sufficient to mediate the inhibition of BCR generated calcium mobilization and cellular proliferation (5, 24). Fc γ RIIA is an activating receptor which associates non-covalently with an accessory subunit called the γ chain which contains a sequence motif ITAM common to antigen receptor of B and T cells (25). These distinct differences in intracytoplasmic domains suggested that different signaling pathways might be triggered by their ligand engagement. The activating receptor is capable of triggering calcium mobilization, degranulation, antibody-dependent cellular toxicity, phagocytosis, cytokine release, and inflammatory responses mediated by macrophages, natural killer cells, neutrophils and MCs (26, 27).

Both lymphoid and myeloid cells, including B cells, T cells, MCs, neutrophils, and macrophages express Fc γ RIIB (28-31). It appears that Fc γ RIIB displays separable inhibitory activities related to MC proliferation and activation. The study in murine bone marrow derived MC and rat MC/basophilic RBL-2H3 cell line showed that coaggregating Fc γ RIIB to Fc ϵ RI could inhibit IgE-induced MC degranulation, and that this inhibition is dependent on the intactness of ITIM domain in the intracytoplasmic tail of Fc γ RIIB (30, 32). A novel chimeric fusion protein consisting of the Fc portion [constant heavy region 2 (CH2) and CH3] of IgG1 and the Fc portion (CH2, CH3, and CH4) of IgE, inhibited antigen-driven histamine release by RBL-2H3, at least in part by blocking IgE-mediated tyrosine phosphorylation of Syk *in vitro*. The same protein was able to block MC recruitment, degranulation, and passive cutaneous anaphylaxis in response to allergens in transgenic mice in which the Fc ϵ RI gene was replaced by the human Fc ϵ RI α chain (33). More recently, Fc γ RIIB was shown to down-regulate the human MC functional response to Fc ϵ RI using the human umbilical cord blood derived MCs (CBMCs). This inhibition was mediated at least in part by changes in protein tyrosine phosphorylation, including marked inhibition of Syk phosphorylation.

Additional studies suggested that phosphorylation of Grb2, adaptor protein Docking Protein 1 (DOK1), and SHIP is associated Fc γ RIIB-mediated inhibition of Fc ϵ RI responses in MCs (34).

Fc γ RIIB can also negatively regulate c-kit dependent MC proliferation. Early studies that investigated the suppressive effects of antibodies on the immune response showed that F(ab')₂ fragments of anti-immunoglobulin antibodies could trigger spleen B cells to proliferate by blocking the binding site of Fc γ RIIB (35). These data suggested that antibodies might affect not only B cell activation but also B cell proliferation. The study in murine MCs showed that anti-c-kit Abs mimicked the effect of kit ligand and induced thymidine incorporation in Fc γ RIIB^{-/-} MCs, but not in wild type MCs unless Fc γ RIIB was blocked or an anti-c-kit F(ab')₂ was used. Inhibition of cell proliferation was observed by coaggregation of c-kit with intact antibodies in wild type MCs and the inhibition correlated with an arrest of cells in G1 phase during the cell cycle. The coaggregation of c-kit with Fc γ RIIB induced the tyrosyl-phosphorylation of Fc γ RIIB, which selectively recruited SHIP1 but not SHIP2. Fc γ RIIB-mediated inhibition of c-kit-dependent cell proliferation was later demonstrated in SHIP1-deficient MCs (36, 37).

Recent development of FcR-deficient mice has made it possible to document a link between FcR signaling and autoimmune diseases. Fc γ RIIB-deficient mice are susceptible to induced autoimmune diseases, such as collagen-induced arthritis and Goodpasture syndrome. In addition, these mice spontaneously develop lupus in a strain specific manner. In a murine model of EAE, Fc γ RIIB deficient mice developed more severe disease than wild-type controls (38, 39).

In addition to Fc γ RIIB, MCs also express a number of other ITIM containing inhibitory receptors that function to regulate the amplitude of the MC antigen receptor response. The common aspect of these receptors is that they are often constitutively phosphorylated on their ITIM sequences and have broad ligand specificities. These receptors include Siglec family, MAFA, and SIRP α .

Sialic acid-binding immunoglobulin-like lectin (Siglec) family

The immunoglobulin superfamily is composed of a large number of cell surface proteins that play a vital role not only in immunity, but also in controlling the behavior of cells in different tissues through their abilities to mediate cell surface recognition events. Siglecs are a recently defined subset of this superfamily. This group of adhesion receptors contains variable numbers of extracellular C2-set Ig domains (two to seventeen) and a unique N-terminal variable region (V-set) Ig domain that confers the ability to bind to sialic acid containing carbohydrate groups (sialosides) of glycoproteins and glycolipids. With the exception of Siglec1 and 4, the cytoplasmic domains of the other 9 Siglecs contain one or more ITIM or ITIM-like motifs that are often dynamically phosphorylated and are characteristic of accessory proteins that negatively regulate transmembrane signaling of cell surface receptor proteins.

Table 2. The genes represent 11 functional Siglecs.

Siglec	Alternative name	Ig domains	ITIM	Expression cell types	Location
Siglec1	Sialoadhesin CD169	17		Macrophages	20p13
Siglec2	CD22	7	4	B cells, Basophils?	19q13.1
Siglec3	CD33	2	2	Myeloid progenitors, Monocytes, DCs, Macrophages	19q13.4
Siglec4	Myelin associated glycoprotein	5		Oligodendrocytes, Schwann cells	19q13.1
Siglec5	OB-BP2, CD33L2	4	1	B cells, Monocytes, Neutrophils, Macrophages, MCs/basophils?	19q13.3
Siglec6	OB-BP1, CD33L, CD33L1	3	1	B cells, Trophoblasts, MCs/basophils	19q13.3
Siglec7	P75/AIRM1, QA79	3	1	CD8 ⁺ T cells, NK cells, Monocytes, DCs	19q13.3
Siglec8		3	1	Eosinophils, MCs/basophils	19q13.33-q13.41
Siglec9		3	1	CD8 ⁺ T cells, B cells, NK cells, Monocytes, Neutrophils	19q13.41
Siglec10		5	2	B cells, NK cells, Monocytes, Eosinophils, DCs	19q13.3
Siglec11		5	1	Macrophages, Microglia	19q13.33

These properties suggest that Siglecs may be involved in cell-cell interactions and regulation of cellular activation within the immune system resulting in intracellular inhibitory signals (Table 2).

The initial discovery of this lectin family came about through independent studies on a sialoadhesin (Siglec1), a macrophage lectin-like adhesion, as well as on the studies of Siglec2 and 3. Both Siglec2 and 3 are the members of Ig superfamily restricted on B cell and both play an important role in regulating B cell activation through cell-cell interactions. Over the past several years a cluster of genes, that encode novel Siglecs that are highly related to Siglec3 (CD33), has been identified and mapped to the long arm (q13.4) of chromosome 19 (40-43). This cluster includes Siglec3, 5, 6, 7, 8, 9 and 10 which share a high degree (50-80%) of sequence similarities. Siglecs are mainly expressed in cells of the hematopoietic origin; ten of the eleven known Siglec family members are expressed among the cell lineages of the hematopoietic system while only two are expressed in neuronal cells. Both B cells and monocytes express a relatively large number of Siglecs, for instance, Siglec2, 5, 6, 9, 10 are expressed on the surface of B cells, while Siglec3, 5, 7, 9, 10 are expressed in monocytes. Siglec9 is broadly expressed in at least five different cell types: neutrophils, monocytes, NK cells, B cells, and subsets of CD8 positive T cells. It is of interest to note that a relatively high level of expression of Siglec5, 6 and 8 proteins can be detected on MC surface during allergic inflammation (43-45). The long form of Siglec8 consists of three Ig domains in the extracellular domain and contains one ITIM motif in the cytoplasmic tail. Interestingly, Siglec8 appears to be preferentially expressed in eosinophils, MCs and basophils. Although it has not been reported whether Siglec5, 6 or 8 inhibit FcεRI mediated MC degranulation, the fact that cross-linking of Siglec8 by an antibody can dampen cell activation and reduce eosinophil viability through induction of apoptosis (46) suggest that these receptors may provide an endogenous negative signal in MCs *in vivo*.

Mast cell function-associated antigen (MAFA)

MAFA is also known as killer cell lectin-like receptor subfamily G member 1 (KLRG1) which is a type II transmembrane protein containing a C-type lectin carbohydrate recognition domain, an intracellular ITIM-like motif, and 4 potential N-glycosylation sites (47-49). Human MAFA gene contains 5 exons with 2 alternatively spliced variants. One lacks exon 3 (E3-minus) and the other lacks both exons 3 and 4 (E3/4-minus). E3-minus MAFA is predicted to have a short extracellular region and its expression appears to be restricted to lung MCs and basophils (49, 50). Activities of MAFA have been studied mostly using rat RBL-2H3 cell line. Studies using immunofluorescence, fluorescent resonance energy transfer and time-resolved phosphorescence anisotropy measuring the rotational mobility of both MAFA and FcεRI provided evidence for the direct interaction of MAFA and FcεRI (51-53). MAFA clustering by its specific monoclonal antibody has been shown to inhibit both the FcεRI-mediated hydrolysis of phosphatidyl phosphoinositides and the transient rise in the intracellular concentration of free calcium ion resulting in suppressed secretory responses and cytokine synthesis. MAFA clustering however did not affect degranulation induced by the Ca²⁺ ionophore A23187 which bypass the receptor and the upstream signaling event, suggesting that inhibition mediated by MAFA may be due to coupling to a cascade upstream of PLC-γ activation (54). This inhibitory action is initiated by tyrosine phosphorylation of MAFA's ITIM motif through SHP2 or SHIP but not SHP1. Interestingly the MAFA mediated inhibition of the activation of the activating receptor (FcεRI) does not require colligation of the activating and inhibitory receptors (55).

Signal regulatory protein α (SIRPα)

SIRPα, also named protein-tyrosine phosphatases non-

receptor type substrate 1, comprises a novel transmembrane glycoprotein family involved in receptor tyrosine kinase-coupled signaling pathways (56). Structurally SIRPs are a member of the Ig superfamily with 3 Ig-like domains in the extracellular region (57, 58). SIRPs can be classified into 2 groups: SIRP α and SIRP β . SIRP α has a long intracellular domain containing four tyrosine residues that form two ITIMs, which recruit SHP-2 and SHP-1 and negatively regulate signal transduction cascades. SIRP β contains only a short cytoplasmic domain and lacks the inhibitory ITIM. In contrast to SIRP α , SIRP β functions as an activating receptor through its interaction with a small adapter protein DAP12 that contains ITAM (59-61). The ligand for human SIRP α has been identified and found to be CD47, an integrin-associated protein (62). Thus, structurally related SIRP α and SIRP β molecules represent a pair of inhibitory and activating receptors.

SIRP α is one of at least 15 members of this SIRP family and the expression of SIRP α is largely restricted to myeloid cells (i.e., macrophages, monocytes, granulocytes, dendritic cells and myeloid progenitors), hematopoietic stem cells, and neurons (62, 63). SIRP α plays an important role during T cell activation and in the regulation of induction of antigen-specific cytotoxic T-lymphocyte responses by dendritic cells mediated by its ligand CD47 (64). The studies of the signaling events associated with leukocyte-epithelial adhesive processes demonstrated the role of SIRP α and CD47 interactions in regulating the rate of neutrophil trans-epithelial migration. In addition, CD47-SIRP α regulates Fc γ and complement receptor-mediated phagocytosis (65-67). Recent studies found that SIRP α is expressed in humans cord blood derived MCs as well as HMC-1 cell line (68) and it also inhibits MC degranulation when colligated with Fc ϵ RI. A chimeric molecule having the transmembrane and intracytoplasmic domains of SIRP α can decrease phosphorylation of Fc ϵ RI ITAMs, reduce the mobilization of intracellular Ca²⁺, the influx of extracellular Ca²⁺, as well as the activation of MAP kinases Erk1 and Erk2, resulting in inhibition of IgE induced MC mediator release as measured by serotonin secretion and cytokine synthesis measuring by TNF α release (68). The SIRP α mediated Inhibition requires the coaggregation of SIRP α chimera with ITAM-bearing high affinity IgE receptors.

Although SIRP α is known to inhibit receptor tyrosine kinase-coupled signaling pathways, some studies have suggested that it can also positively regulate the mitogen-activated protein kinase (MAPK) pathway in response to insulin and can potentiate integrin-induced MAPK activation (69, 70). This process is mediated by its associated transmembrane adapter protein DAP12 (59, 60).

Leukocyte immunoglobulin-like receptor (LIR) complex

Leukocyte Ig-like receptors (LIRs) are a family of immunoreceptors located on human chromosome 19q13.4 and expressed in a wide variety of leukocytes such as monocytes, B cells, dendritic cells, and natural killer cells. LIR clusters are organized within the region of 1 Mb and contain over 30 Ig superfamily genes that function either as

activating or inhibitory receptors. These receptors differ in their transmembrane and cytoplasmic sequences. The activating Ig-like receptors among the group have a short cytoplasmic tail and a positively charged residue (arginine or lysine) in their transmembrane domains that bind to the negatively charged transmembrane aspartate residues of adaptor molecules (71). On the other hand, the inhibitory receptors among this family lack the positively charged residue in their transmembrane region and have long cytoplasmic tails containing one to four ITIM motifs. Phosphorylation of the tyrosine residues within the ITIMs recruits the Src-homology-2-domain-containing phosphatases and triggers negative signaling to inhibit cellular lysis (72). In human, these negative receptors can be broadly classified into three groups based on the homology of their Ig-like domains, gene architecture and organization. They are the leukocyte Ig-like receptors (LILRs)/Ig-like transcripts (ILTs), killer-cell Ig-like receptors (KIRs), and leukocyte-associated Ig-like receptors (LAIRs) (73).

In an effort to identify LIRs that are expressed in human MCs, a human monocyte and lung cDNA library was screened with probes of mouse inhibitory receptor gp49B1 and LILRB4 was cloned (also named as ILR3, LIR5) (74). In rodent, inhibitory molecule mouse gp49B1 is highly expressed in MCs and is a member of the Ig superfamily with two C2-type Ig-like domains and contains two cytoplasmic ITIMs. The function of gp49B1 has recently been elucidated and gp49B1 has been shown to suppress the LPS-induced increase in intravascular neutrophil adhesion and to provide critical innate protection against a pathologic response to a bacterial component (75, 76). However, there is no strong evidence that LILRB4 is expressed at protein level on human MCs.

Another pair of inhibitory and activating receptors is paired Ig-like receptors (PIRs). The paired Ig-like receptors of activating (PIR-A) and inhibitory (PIR-B) types were originally identified in mice on the basis of limited sequence homology with the human IgA Fc receptor (Fc α R) (77-79). The PIR-B molecule contains an uncharged transmembrane segment and four potential ITIMs in the cytoplasmic tail. When the tyrosine residues are phosphorylated, the ITIMs of PIR-B recruit the protein tyrosine phosphatase SHP-1, and possibly SHP-2 to inhibit cell activation (12, 80-82). Mouse PIR-B has six Ig-like domains and is expressed in a wide variety of hematopoietic cells. MCs derived from mouse bone marrow or splenic progenitors were found to produce the PIR-B and PIR-A transcripts at comparable levels, but interestingly, cell surface protein expression of PIR-A could not be detected whereas PIR-B was easily and consistently detected. It may suggest a striking prevalence of PIR-B on MCs (83). By using rat RBL-2H3 line, inhibitions of exocytosis of granule mediators and calcium flux were observed (81, 82). It is likely that functional homologs of these two receptors in human MCs may be related to LIR1 but are yet to be confirmed in functional studies.

Other inhibitory receptors

Up to date, a relatively small number of inhibitory receptors have been identified on human MCs. Other

well-characterized inhibitory receptors are predominantly expressed in lymphocytes. For instance, cytotoxic T-lymphocyte antigen (CTLA4) plays an important inhibitory role in regulating T cell differentiation by controlling the overall strength of the T cell activation signal (84, 85). CD72 is the human homolog of mouse Lyb2 (86) that is restricted to B-lineage cells and is involved in signals for B cell proliferation. PD-1 may play a role in B cell differentiation during the pro-B cell stage (87). Another Ig superfamily member platelet endothelial cell adhesion molecule (PECAM1) is expressed on the surface of circulating platelets, monocytes, neutrophils, and particular T cell subsets and is known to mediate cell adhesion or antigen recognition for immunoglobulins, T cell receptors, and MHC molecules. More recently, a novel type of inhibition receptor has been demonstrated on mouse MCs. CD200R and its splice variants, which do not possess an ITIM in the cytoplasmic domain, were found to be highly expressed in MCs and bind to adaptor proteins Dok1 and Dok2 upon activation. Subsequently CD200R (88) binds to SHIP and recruits RasGAP that mediates the inhibition of the Ras/MAPK pathways. This novel inhibitory pathway is triggered and activated without the need of co-ligation to an activating receptor. Human homolog of CD200R has been identified but the expression level in human MCs and its role in regulating the function of human MCs remains to be defined (88, 89).

Conclusion

A large number of ITIM containing receptors have been discovered in the past few years and many of them are expressed in myeloid cells of the innate immune system. Several general paradigms of the negative regulation mediated by this class of inhibitory receptors have been defined. When cellular activation is triggered by receptors containing ITAM motif, counteracting inhibitory signals are delivered through ITIM motif containing receptors, which result in the phosphorylation by Src-family protein kinases and the recruitment of either SHIP or SHP-1/SHP-2. The recruited phosphatases inhibit the signaling pathways of activating receptor and eventually raise activation thresholds. The picture of the physiological relevance of these pathways in the immune response has now emerged and the inhibitory molecules play an important role as the activation molecule in immune responses. The significance of these receptors is evidenced by the conservation of ITIM in immune receptors during their evolution especially for the inhibitory IgG Fc receptor FcγRIIB. Human MCs express at least four different types of inhibitory receptors on their surfaces, and yet many others remain to be identified. Although the reason for the co-expression of multiple inhibitory receptors in the same cells is not yet fully understood, it is conceivable that the balances of our immune system depend on many different types of immune responses. This process involves multiple step regulations mediated by different activating and inhibitory control mechanisms. It is not clear whether one ITIM receptor is sufficient for negative control of an activating event. In some instances, concerted coordination of multiple ITIM containing receptors may be desired for

optimal negative regulation. Alternatively different receptors may be activated sequentially during the immune response in order to impose continued suppression. It is also possible that different types of inhibitory receptors may be activated during different types of the immune response.

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